PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Reduced magnetization transfer ratio in cognitively impaired patients at the very early stage of multiple sclerosis: a prospective, multicenter, cross-sectional study
AUTHORS	Faiss, Jürgen ; Dähne, Doreen; Baum, Karl; Deppe, Ralf; Hoffmann, Frank; Köhler, Wolfgang; Kunkel, Annett; Lux, Anke; Matzke, Mike; Penner, Iris-Katharina; Sailer, Michael; Zettl, Uwe

VERSION 1 - REVIEW

REVIEWER	Friedemann Paul Charité University Medicine Berlin, Germany
REVIEW RETURNED	06-Jan-2014

GENERAL COMMENTS	In principle, this is a well written, interesting and clinically relevant study that deserves to be published after some modifications: - what was the inclusion period? - was there a minimum time lapse between onset of symptoms and performance of neuropsychology and MRI? - was any of the patients started on a DMD prior to study inclusion or were all treatment-naive? - can the authors provide information on the clinical symptoms of the qualifying clinical event, e.g. optic neuritis etc.? this may be relevant as they state that patients with severe motor or visual impairment were excluded? were there patients who had to be excluded because they had incomplete recovery from their first relapse? if so, how many? - data evaluation and analysis: the authors should state that this was an exploratory pilot study with no sample size calculation which justifies the lacking correction for multiple comparisons - methods/results: it is not clear to me what cut-off the authors defined for fatigue versus non-fatigue on the MFIS. The percentage of 36 with fatigue is far lower than in many other studies. The authors state that fatigue was not related to cognitive impairment which is in contrast to PMID: 20610494, please discuss. What was the cut-off for depression? - table 2: it is no clear to me what the authors mean by T1-w lesion load, is this the volume (in what?) of contrast-enhancing lesions or the volume of T1 hypointense (black hole) lesions? How many patients had contrast enhancement on their MRI and did this influence cognitive performance? See for example PMID: 19917984. - table 3: from the data presented it could be assumed that fatigue and depression were more prevalent in the cognitively impaired group, what does statistics say? Please provide percentages of fatigued and depressed patients for the two subgroups as well - discussion: -- on fatigue, depression and cognitive impairment see above, please extend discussion accordingly

	-- "neuropsychological assessment should not be done during a relapse or tratment with corticosteroids..." PMID: 19917984 and PMID: 18337428 apply here, please extend discussion accordingly --was there an association between cognitive performance and premorbid education? See recent discussion on cognitive reserve PMID: 23576622 --the lack of correlation between EDSS and cognition may in part be explained by spinal cord pathology, please discuss -- as the entire BRB-N is quite time-consuming is there any other shorter test the authors would recommend for use in daily clinical practice?

REVIEWER	Tobias Granberg Karolinska Institutet Karolinska University Hospital
REVIEW RETURNED	12-Jan-2014

GENERAL COMMENTS	This prospective cohort study investigates differences in MTR in patients with a first clinical event suggestive of MS sub-grouped based on cognitive function. The study seems well structured with comprehensive neuropsychological testing and raises awareness for early cognitive impairment in MS and the use of non-conventional MR techniques. Its results imply that MTR could prove to be a surrogate marker for cognitive impairment in this condition, which has been suggested previously in similar studies. There are however aspects of the manuscript that need revision/clarification: Methodology: 1) Although it is stated that the study was conducted in accordance with the Helsinki declaration, it does not specifically say that written informed consent was obtained. 2) The authors mention that the participants were recruited at six MS centers but do not mention where these centers are located nor how many participants each center enrolled. 3) In the manuscript for this multicenter study only one specific model of MRI equipment is mentioned. Were all imaging done on one single MRI scanner? Otherwise, the number of sites and scanners used should be specified. This is especially important in MTR studies were the measurements are heavily reliant on the equipment used. 4) Lesion segmentation was performed using DispImage. The authors do not specify how this method was applied nor reference it. From my understanding, it is a semi-automatic method. What training did the rater(s) have and how was a lesion defined? 5) The authors do not specify by whom the neuropsychological testing was performed. What training did the rater(s) have? 6) The lack of a non-diseased control group is a limitation that should be mentioned. Statistics: 7) The pre-determined alfa level for significance is not specified. However, it can be assumed to be 0.05. 8) It seems as if not all of the 47 patients were included in the analysis of the MTR data (based on t[43]) but no explanation is given as to which patients are missing and why. 9) It is mentioned that there were no significant differences between the subgroups in terms of T1/T2 lesion load but no p-values are given. This could be complemented in Table 2.

	10) Seeing how at least three different MRI measures were used in this study and compared between the subgroups, it is arguable that there should be a correction for the false discovery rate. Linguistics: 11) There are inconsistencies in the number of significant figures used. 12) There are grammatical oversights that impacts the overall impression of the manuscript such as: - The use of different tenses in the same paragraph, i.e. the last sentence on page 6. - Percent is inconsistently written as “percent” or “%”. - Inconsistencies in the use of “.” or “,” as a decimal point. References: 13) There are similar studies regarding the use of MTR in association with cognitive testing in CIS/MS that are not referenced. In order to put the current study and its results in a context, these studies might be of value. In particular previous work from the group in Milan led by professors Amato and Rocca have been fundamental for the area. (I have no conflicts of interests regarding this recommendation).

VERSION 1 – AUTHOR RESPONSE

Reviewer Name Friedemann Paul
Institution and Country Charité University Medicine Berlin, Germany
Please state any competing interests or state ‘None declared’: None declared

In principle, this is a well written, interesting and clinically relevant study that deserves to be published after some modifications:

- what was the inclusion period?
- Patients were included into the study directly after the diagnosis of MS or CIS was established and after checking the inclusion and exclusion criteria.
- was there a minimum time lapse between onset of symptoms and performance of neuropsychology and MRI?
- Patients underwent the neuropsychological tests and MRI between 90 to 180 days after the first symptoms of MS or CIS were detected.
- was any of the patients started on a DMD prior to study inclusion or were all treatment-naive?
- All patients were treatment-naive. DMD prior to study inclusion were defined as exclusion criteria.
- can the authors provide information on the clinical symptoms of the qualifying clinical event, e.g. optic neuritis etc.? this may be relevant as they state that patients with severe motor or visual impairment were excluded? were there patients who had to be excluded because they had incomplete recovery from their first relapse? if so, how many?
- The first clinical symptoms were related to the affected functional system recorded. Patients were divided into two groups: a) with a single functional system affected and b) with multiple functional systems affected – it was not used for later data analysis but to confirm the diagnosis of MS.
- data evaluation and analysis: the authors should state that this was an exploratory pilot study with no sample size calculation which justifies the lacking correction for multiple comparisons
- This section will be included into the manuscript.
- methods/results: it is not clear to me what cut-off the authors defined for fatigue versus non-fatigue on the MFIS. The percentage of 36 with fatigue is far lower than in many other studies. The authors state that fatigue was not related to cognitive impairment which is in contrast to PMID: 20610494, please discuss. What was the cut-off for depression?

- The Modified Fatigue Impact Scale (MFIS) consists of 21 statements regarding to fatigue, cut-off is a total value above 22 points. The percentage of 36 with fatigue may be lower than in other studies because of the short disease duration and the high degree of CIS-patients at the time of data evaluation (77% of the sample was classified as CIS and 23% showed a relapsing-remitting course (RRMS)) and the mildly disabled sample size(Median EDSS score was 1.5). The lack of correlation between cognition and fatigue in contrast to result in other studies could be explained by the fact that our patients were in a very early stage of the disease and were disabled to a very low degree.

Depressive symptoms were assessed using a german version of “Center for Epidemiological Studies” Depression Scale (ADS-L). Cut-off was a total value above 23 points.
- table 2: it is no clear to me what the authors mean by T1-w lesion load, is this the volume (in what?) of contrast-enhancing lesions or the volume of T1 hypointense (black hole) lesions? How many patients had contrast enhancement on their MRI and did this influence cognitive performance? See for example PMID: 19917984.
- The T1-w lesion load is related to the volume of T1 hypointense lesions.
- table 3: from the data presented it could be assumed that fatigue and depression were more prevalent in the cognitively impaired group, what does statistics say? Please provide percentages of fatigued and depressed patients for the two subgroups as well
- T-test-analysis showed no significant differences regarding the occurrence of depression or fatigue between the two subgroups (see section “results” of the manuscript). In the cognitively impaired subgroup 4.3% were depressed and 21.3 % fatigued, in the cognitive preserved subgroup 6.3 % were depressed and 14.9 % fatigued.
- discussion:
- on fatigue, depression and cognitive impairment see above, please extend discussion accordingly
- Depression and fatigue are common symptoms in MS-patients which can negatively affect the performance on neuropsychological tests. In our study population, neither depression nor fatigue showed a significant correlation to test performance but only a relative low percentage of our patients complained of clinical significant depression and fatigue. This might be related to the short disease duration and the relatively mild physical disability (EDSS) of our study population.
- "neuropsychological assessment should not be done during a relapse or tratment with corticosteroids..." PMID: 19917984 and PMID: 18337428 apply here, please extend discussion accordingly
- References and discussion were expanded. Recent research show, that the definition of relapse is difficult and might represented only by fatigue or Gd enhanced lesions without clinical symptoms, both could show a negative impact on test performance (Flachenecker P, Meissner H. Fatigue in multiple sclerosis presenting as acute relapse: subjective and objective assessment. Mult Scler 2008;14(2):274-7; Bellmann-Stobl J, Wuerfel J, Aktas O et al. Poor PASAT performance correlates with MRI contrast enhancement in multiple sclerosis. Neurology 2009; 73(29):1624-7).
-was there an association between cognitive performance and premorbid education? See recent discussion on cognitive reserve PMID: 23576622
- A recognition vocabulary test (WST) was used to assess the pre-morbid intelligence to consider the possible positive effect of “cognitive reserve” on test performance. In our study population pre-morbid intelligence was averaged and between the two subgroups (cognitive impaired and preserved patients) no significant differences were obtained (table 3 was expanded).
-the lack of correlation between EDSS and cognition may in part be explained by spinal cord pathology, please discuss
- Previous studies often described a lack of correlation between EDSS and cognition. This might be explainable as EDSS is not sensitive in determine cognitive disorders and physical and cognitive impairment can independently from each other occur during the disease course. Potentially, cognitive impairment is a first sign of the disease. Our study population was only mildly disabled measured by EDSS and had short disease duration. Cognition might be a sensitive marker especially at the onset of the disease.

- as the entire BRB-N is quite time-consuming is there any other shorter test the authors would recommend for use in daily clinical practice?
- Recently, an expert consensus committee of MS-specialists have recommended a Brief International Assessment of Cognition for MS (BICAMS), which takes 15 minutes to complete, requires no special equipment and comprises the SDMT, a learning test (The California Verbal Learning Test) and a visuospatial memory test (The Brief Visuospatial Memory Test-Revised). In our opinion these test battery can recommend for the use in daily clinical practice unless extensive cognitive analysis is not possible. (Langdon DW et al., Recommendations for a Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS). Mult Scler J 2012; 18(6): 891-898.)
- references: see my comments above

Reviewer Name Tobias Granberg
Institution and Country Karolinska Institutet
Karolinska University Hospital
Stockholm, Sweden
Please state any competing interests or state ‘None declared’: None declared

This prospective cohort study investigates differences in MTR in patients with a first clinical event suggestive of MS sub-grouped based on cognitive function. The study seems well structured with comprehensive neuropsychological testing and raises awareness for early cognitive impairment in MS and the use of non-conventional MR techniques. Its results imply that MTR could prove to be a surrogate marker for cognitive impairment in this condition, which has been suggested previously in similar studies. There are however aspects of the manuscript that need revision/clarification:

Methodology:
1) Although it is stated that the study was conducted in accordance with the Helsinki declaration, it does not specifically say that written informed consent was obtained.
- Written informed consent was obtained prior study inclusion.
2) The authors mention that the participants were recruited at six MS centers but do not mention where these centers are located nor how many participants each center enrolled.
- Patients were recruited at six MS centers (Halle (N=11), Henningsdorf (N=6), Magdeburg (N=5), Rostock (N=10), Teupitz (N=7) and Wermsdorf (N=8)).
3) In the manuscript for this multicenter study only one specific model of MRI equipment is mentioned. Were all imaging done on one single MRI scanner? Otherwise, the number of sites and scanners used should be specified. This is especially important in MTR studies were the measurements are heavily reliant on the equipment used.
- MRI was conducted for all patients at the MS-center Magdeburg.
4) Lesion segmentation was performed using DispImage. The authors do not specify how this method was applied nor reference it. From my understanding, it is a semi-automatic method. What training did the rater(s) have and how was a lesion defined?
- Lesion volume quantifcation was performed on T2 - and on T1 weighted images using a semi-automated, local contour technique. T1 lesions were identifed as hypointense areas on the T1 weighted scans and confirmed on a T2-weighted scan as lesions. The semi-quantitative lesion load measurement was performed using a highly reproducible (Molyneux et al.,1998) threshold technique based upon the local environmental intensity of the lesion (DispImage software package supplied by Dave Plummer, University College London, UK). (Molyneux PD, Tofts PS, Fletcher A, Gunn B, Robinson P,Gallagher H, et al.
Precision and reliability for measurement of change in MRI lesion volume in multiple sclerosis: a comparison of two computer assisted techniques.
J Neurol Neurosurg Psychiatry 1998; 65: 42-7.

5) The authors do not specify by whom the neuropsychological testing was performed. What training

did the rater(s) have?
- Neuropsychological tests were performed by neuropsychologists. The used test-procedure was selected by means of high objectivity (independence of rater). Before study start, neuropsychologists were instructed in using the same test-order and instructions to patients.
6) The lack of a non-diseased control group is a limitation that should be mentioned.
- A non-diseased control group was included to control practice effects. A follow-up after 18 months will be published later. Therefore, control group data was not included in this manuscript.
Statistics:
7) The pre-determined alfa level for significance is not specified. However, it can be assumed to be 0.05.
- A significance alfa level of 0.05 for minimum was pre-determined.
8) It seems as if not all of the 47 patients were included in the analysis of the MTR data (based on t[43]) but no explanation is given as to which patients are missing and why.
- All of the 47 patients underwent the neuropsychological tests but 1 patient did not got a MRI-investigation, 1 other patient had no MTR data, MTR data based on 45 patients (21 patients were cognitively preserved, 24 impaired).
9) It is mentioned that there were no significant differences between the subgroups in terms of T1/T2 lesion load but no p-values are given. This could be complemented in Table 2.
- T1 and T2 lesion load was completed in table 2, p-value for T1 was t(44)= -0.186, p= 0.854 n.s. and p-value for T2 was t(44)= -0.365, p=0.717 n.s.
10) Seeing how at least three different MRI measures were used in this study and compared between the subgroups, it is arguable that there should be a correction for the false discovery rate.
- This correction was not calculated.
Linguistics:
11) There are inconsistencies in the number of significant figures used.
- was not found
12) There are grammatical oversights that impacts the overall impression of the manuscript such as:
- The use of different tenses in the same paragraph, i.e. the last sentence on page 6.
- will be corrected
- Percent is inconsistently written as “percent” or “%”.
- was changed in “%”
- Inconsistencies in the use of “.” or “,” as a decimal point.
- was changed in “.”
References:
13) There are similar studies regarding the use of MTR in association with cognitive testing in CIS/MS that are not referenced. In order to put the current study and its results in a context, these studies might be of value. In particular previous work from the group in Milan led by professors Amato and Rocca have been fundamental for the area. (I have no conflicts of interests regarding this recommendation)- will be considered, if available

VERSION 2 – REVIEW

REVIEWER	Friedemann Paul Charité University Medicine Berlin, Berlin, Germany
REVIEW RETURNED	14-Feb-2014

GENERAL COMMENTS	The manuscript has substantially improved. There are only a few minor corrections that should be made before publication: - results: 23% of patients were later classified as RRMS, according to which diagnostic criteria? McDonald 2001/2005/2010? Please specify. - discussion p35: "....a visuospatial memory test can BE recommended"

	- "the lack of correlations...." PMID:20610494 and PMID:19796282 apply here - please rephrase the last part of the sentence "...only minor physical disabled" - discussion "this can be explained..." better: this can be explained by the fact that EDSS...." - please clearly state in table 2 that t1w lesion load refers to black holes and not Gd lesions The paper does not need to be re-reviewed.

REVIEWER	Tobias Granberg Karolinska Institutet Karolinska University Hospital
REVIEW RETURNED	16-Feb-2014

GENERAL COMMENTS	The authors have thoroughly revised the manuscript in accordance with given comments. The manuscript is solid and suitable for publication. I have no further critique.

VERSION 2 – AUTHOR RESPONSE

Reviewer Name Friedemann Paul
Institution and Country Charité University Medicine Berlin, Berlin, Germany
Please state any competing interests or state ‘None declared’: None declared

The manuscript has substantially improved. There are only a few minor corrections that should be made before publication:
- results: 23% of patients were later classified as RRMS, according to which diagnostic criteria? McDonald 2001/2005/2010? Please specify.
- MCDonalds critera 2001 were used
- discussion p35: "....a visuospatial memory test can BE recommended"
- BE was included
- "the lack of correlations...." PMID:20610494 and PMID:19796282 apply here
- references were included
- please rephrase the last part of the sentence "...only minor physical disabled"
- sentence was removed because it had no substantial new information
- discussion "this can be explained..." better: this can be explained by the fact that EDSS...."
- was changed according to your suggestion
- please clearly state in table 2 that t1w lesion load refers to black holes and not Gd lesions
- was included (table 2)

The paper does not need to be re-reviewed. The paper should be published after the few minor modifications suggested above

Reviewer Name Tobias Granberg
Institution and Country Karolinska Institutet
Karolinska University Hospital
Stockholm, Sweden
Please state any competing interests or state ‘None declared’: None declared

The authors have thoroughly revised the manuscript in accordance with given comments. The manuscript is solid and suitable for publication. I have no further critique.